Discovery of a cryptic species of *Heptathela* from the northernmost part of Okinawajima Is., Southwest Japan, as revealed by mitochondrial and nuclear DNA

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Abstract — Heptathela spiders of Okinawajima Is. were taxonomically revised using DNA sequence data. An analysis of mt-COI gene sequences revealed two clades (HY and HH), one of which (HY) was identified as Heptathela yanbaruensis because HY included specimens from Yona, the type locality of this species. To evaluate gene flow between HY and HH, the sequences of the nuclear 28S-rRNA and histone H3 genes were analyzed for specimens collected from two sympatric localities. In the two nuclear genes of HY and HH, all specimens had 5 homozygous positions with bases that differed between HY and HH. This indicates that there has been no gene flow between HY and HH, which is solid evidence for their reproductive isolation. Thus, it was concluded that HY and HH should be recognized as independent species, and HH is described as a new species under the name Heptathela helios n. sp. There were no good morphological diagnostic features other than the body size of the adult males of the two species (yanbaruensis < helios); thus, females of these two species can be distinguished only by DNA sequence data.

Key words — *Heptathela helios*, new species, reproductive isolation, species delimitation, species boundary, DNA, nuclear

Introduction

A total of nine species have been described from Japan in the genus Heptathela (Ono 2009) and one of these species, Heptathela yanbaruensis Haupt 1983, is found on Okinawajima Is. However, previous studies may have underestimated the true diversity of this genus because spiders with limited dispersal capabilities may contain cryptic species without distinctive morphological characters. In fact, DNA sequence data showed that Aptostichus simus, a trapdoor spider with limited dispersal capability, is geographically subdivided and divergent in the absence of morphological differentiation (Bond et al. 2001). A similar geographical divergence was detected in Ryuthela (Tanikawa 2013a, b), a sister group of Heptathela. Therefore, we collected many specimens of Heptathela yanbaruensis from its entire distribution range and looked for the presence of cryptic species using DNA sequence data.

This study was performed in the following three steps. First, we examined partial sequences of the mitochondrial cytochrome oxidase subunit I (mt-COI, DNA barcoding gene) from all the specimens, with the goal of identifying phylogenetic groups and their distribution ranges. Second, collected many specimens from localities in which spiders of different phylogenetic groups are sympatric and analyzed

partial sequences of the nuclear 28S-rRNA (28S) and nuclear histone H3 (H3) genes to examine potential gene flow. Finally, we investigated possible morphological differences between the groups.

Materials and methods

Sampling. Heptathela specimens were collected from the northern part of Okinawajima Is. (Fig. 1), which is the distribution range of *H. yanbaruensis* (Haupt 2003). To cover the whole distribution range, collection was conducted in a wider area than the previously defined range. Three or more adult spiders per locality were generally collected, but juvenile spiders were also included if adults were not available in sufficient numbers. Female and juvenile specimens were preserved in 99.5% ethanol at 4°C. Male adult spiders were preserved in 75% ethanol at room temperature, but the right fourth leg was dissected and preserved in 99.5% ethanol at 4°C for molecular analysis. Young males were brought back alive to the laboratory and reared until becoming adults.

DNA extraction, polymerase chain reaction and sequencing. Genomic DNA was extracted from muscle of legs of large individuals or the whole cephalothorax of small individuals using DNeasy Blood & Tissue kit (Qiagen, Inc., Germantown, MD) or FavorPrep Tissue

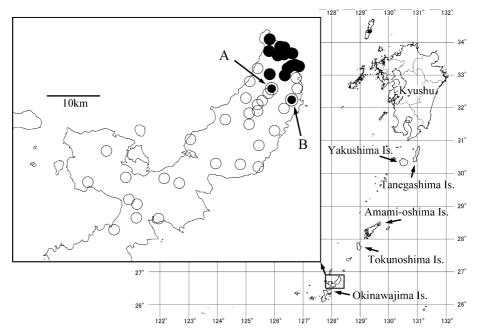


Fig. 1. Collection localities of *Heptathela yanbaruensis* and *H.* sp. Specimens were identified by mt-COI sequence data. Open circle: *H. yanbaruensis*; filled circle: *H.* sp.; arrows A and B: sympatric localities. *H.* sp. is described as *H. helios* n. sp. in this study.

Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp., Ping-Tung). Mt-COI was amplified using the primer combination CB1: 5'-TAT GTA CTA CCA TGA GGA CAA ATA-3' (Jermiin & Crozier 1994) with HCOI-2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer et al. 1994). The reactants were initially denatured for 2 min at 90°C, proceeded with 40 cycles of 15 sec at 90°C, 20 sec at 50°C, 4 min at 72°C. 28S was amplified using the primer combination ZX1: 5'-ACC CGC TGA ATT TAA GCA TAT-3' with AS8OP1: 5'-AGA GCC AAT CCT TGT CCC GA-3' (Bond & Hedin 2006). The reactants were initially denatured for 2 min at 94°C, proceeded with 40 cycles of 30 sec at 94°C, 20 sec at 50°C, 2 min at 72°C. H3 was amplified using the primer combination H3aF: 5'-ATG GCT CGT ACC AAG CAG ACV GC-3' with H3aR: 5'-ATA TCC TTR GGC ATR ATR GTG AC-3' (Colgan et al. 1998). The reactants were initially denatured for 2 min at 94°C, proceeded with 30 cycles of 30 sec at 94°C, 20 sec at 50°C, 15 sec at 72°C. PCR products were purified using the ExoSAP-IT (GE Healthcare Bio-Sciences, Co. Ltd., Buckinghamshire, England). The purified PCR products were sequenced using the BigDye terminator cycle sequencing kit (ver.3.1) using the primer HCOI-2198 (mt-COI), ZX1 (28S), or H3aF (H3) and analyzed on ABI 3100 or ABI 3130xl automated DNA sequencer (Applied Biosystems, Foster City, CA). Chromatograms were checked by eye using MEGA version 5.05 (Tamura et al. 2011). Sequence alignments were done by MUSCLE (Edgar 2004) in MEGA. Overall mean pdistance was calculated using MEGA by averaging the number of base differences per site over all sequence pairs. The phylogenetic tree was constructed by Neighbor Joining

method using MEGA.

Morphology. The morphological characters were examined under stereoscopic microscope, M3Z (Wild Heerbrugg AG, Heerbrugg, Switzerland), photographs were taken by EOS D60 or EOS Kiss X7 with EF100 mm macro lens and MT-24EX macro twin flash (Canon Inc., Tokyo, Japan) or attached to stereoscopic microscope. The relationship between width of carapace and distance from sympatric localities were compared for checking the occurrence of "character displacement". All measurements were given in mm.

Deposit. Nucleotide sequence data are available in the DDBJ/EMBL/GenBank databases. The type specimens designated in this study are deposited in the collection of the Department of Zoology, National Museum of Nature and Science, Tokyo (NSMT).

Results

In total, 210 *Heptathela* specimens from 47 localities (Fig. 1, Appendix) were sampled and mt-COI partial sequences of 578 bp were obtained. Haplotype names and accession numbers are shown in the Appendix. In the unrooted NJ tree, two clades were detected (Fig. 2A, B). Specimens in clade A were identified as *Heptathela yanbaruensis* (HY) because this clade included specimens from Yona, the type locality of the species. The remaining specimens (those of clade B) were designated as the spiders in question (HH). Of the 210 collected specimens, 175 were identified as HY and 35 as HH. The uncorrected p-distances (number of base differences per site) were 0.002–0.130 among HY, 0.002–0.106 among HH, and 0.135–0.196 between HY and HH. The distribution range of HH was

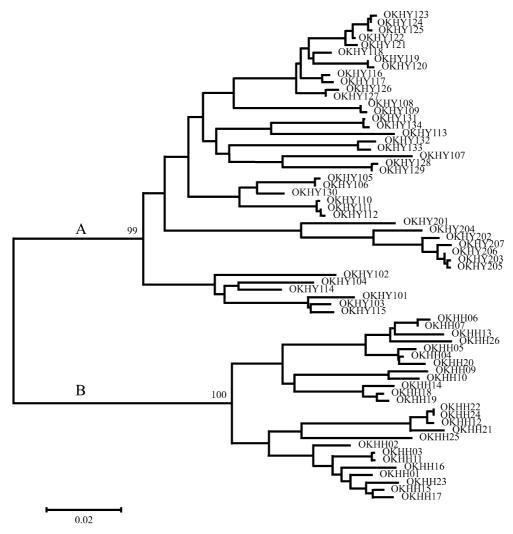


Fig. 2. Unrooted bootstrap consensus tree obtained by the Neighbor Joining method for the specimens examined. Scores at two nodes are bootstrap values (1000 replicates). Specimens of clade A are identified as *Heptathela yanbaruensis* and those of clade B as *H. helios* n. sp. in this study. The scale bar shows the substitution per site.

limited to the northernmost part of Okinawajima Is. (Fig. 1).

A total of 63 specimens were obtained from two sympatric localities (Fig. 1A, B), of which 58 individuals were determined to be HY and 5 to be HH based on the mt-COI sequence. In the nuclear 28S partial sequence (627 bp), all the 58 HY individuals were homozygous for T at positions 422 and 603, while all 5 HH individuals were homozygous for C. In the nuclear H3 sequence (303 bp), all 58 HY individuals were homozygous for C at positions 103 and 196, and G at position 235; while all 5 HH individuals were homozygous for T at positions 103 and 196, and A at position 235.

No morphological gaps were found in the male palpal structure of HY (Fig. 4H–J) and HH (Fig. 4E–G), which is the only useful morphological feature for identification of *Heptathela* spiders. The only clear morphological difference between HY and HH was the body size of adult males: the range of the carapace width of adult male HY specimens (n=15) was 3.48-4.25, while that of HH (n=5) was 4.63-

5.50 (Fig. 3B), though the male HH specimens used in this study were only 5. The difference between the two was significant (P < 0.005, Mann-Whitney U-test). In female specimens, the average carapace width of HY (3.80, n = 61) was smaller than that of HH (4.86, n = 8), but the ranges overlapped to some extent (HY, n = 61: 3.08–4.75, HH, n = 8: 4.38–5.89) (Fig. 3A). The difference between HY and HH were significant again (P < 0.001, Mann-Whitney U-test).

Discussion

Two nuclear genes of HY and HH had 5 positions where all the specimens exhibited homozygosity, but the bases of HY and HH were different. If intercross between HY and HH had occurred, these positions should have been heterozygous. Therefore, there has been no gene flow between HY and HH, which is strong evidence for their reproductive isolation. Consequently, it is concluded that HY and HH are independent species and HH is described as a new species in the following section.

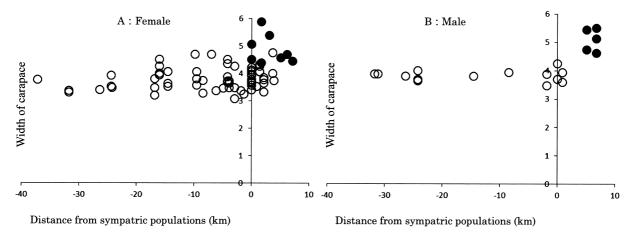


Fig. 3. Relationship between the carapace width (mm) and the distance from sympatric populations (km); + means northeast direction and — means southwest direction. A: female, B: male. Open circles: HY (*Heptahela yanbaruensis*), solid circles: HH (described as *H. helios* n. sp in this srudy).

A previous study (Haupt 2003) revealed that females of *Heptathela* or related spiders attract males with a kind of pheromone and that males produce vibrational signals using their palps when approaching female burrows. Therefore, reproductive isolation between HY and HH may be retained by the difference in chemical substances made by females and/or the difference in male courtship signals. The mating season of these two species may also differ because mature HY males were found in late October to early December, while no adult HH males were found in this season. All the mature male specimens of HH used in this study were collected as juveniles and reared until becoming adults, therefore the maturation period of HH males is still unknown.

The large p-distance for the mt-COI sequences between HY and HH suggests a long history after speciation of these two species. The distribution range of HH is currently restricted to the northernmost part of Okinawajima Is., but this range may have been wider in the past, and probably northward, when the land area was larger than present.

Distributional map of HH and HY indicates that the distributional zone of overlap between the two is rather narrow (Fig. 1). It is likely that selective forces in evolution of some morphological characters is different between sympatric populations where HH and HY co-occur and allopatric populations where only one of the two forms occurs if there is some interspecific competition between HH and HY. In this sense, it is interesting that female body size of HY seems to be decreased in the sympatric zones, while that of HH appears to be increased in the same zone than nearby non-sympatric populations of respective species (Fig. 3A). Thus, it seems that this situation in the zone of overlap indicates occurrence of "character displacement" in female body size of the two species, although, unfortunately, we have no solid answer whether this phenomenon is observed also in males since no adult males of HH have been obtained from the sympatric populations (Fig. 3B).

Description of New Species *Heptathela helios* n. sp. [Japanese name: Kunjan-kimuragumo] (Fig. 4A-G)

Type series. All the type specimens were collected from Kunigami-son, Okinawajima Is., Japan, by Akio Tanikawa unless noted otherwise. Holotype: 18, Oku (N26.84274 E128.28737), 26-V-2010 (collected as juvenile and became adult on 9-IX-2012) (NSMT-Ar 12851). Paratypes: 28, same data as holotype (became adults on 15-X-2010 and 5-XII-2012) (NSMT-Ar 12852-12853); 1², Oku (N26.83633 E128.27544), 30-V-2007 (NSMT-Ar 12854); 28, Oku (N26.83436 E128.27615), 26-V-2010 (collected as juveniles and became adults on 20-X-2010 and 13-I-2013) (MSNT-Ar 12855-12856); 1° , Oku (N26.83436 E128.30238) (NSMT-Ar 12857), 2², Benoki (N26.79985 E128.26064), 10-XII-2012 (NSMT-Ar 12858-12859); 1⁹, Benoki (N26.79797 E128.28802), 11-XII-2012, Booppa Petcharad leg. (NSMT-Ar 12860); 1° , Ada (N26.75557 E128.30072), 13-VI-2006 (NSMT-Ar 12861), 1², same locality, 11-XII-2012 (NSMT-Ar 12862).

Etymology. Arbitrary naming. Helios is the personification of the sun in Greek mythology. "Kunjan" of the Japanese name is an alias name of "Kunigami" which refers to the northern part of Okinawajima Island.

Diagnosis. This species can be distinguished from *H. yanbaruensis* by the following unique combinations of mt-DNA COI gene nucleotide site substitutions; C (21), C (262), C (266), A (384), C (558). The body size of adult males of *H. helios* is larger than that of *H. yanbaruensis*, but the male palps of these two species are quite similar (compare Fig. 4E–G and 4H–J), which cannot be used for identification. Females of *H. helios* also cannot be distinguished from *H. yanbaruensis* by morphological features.

Description. Based on the holotype & (NSMT-Ar

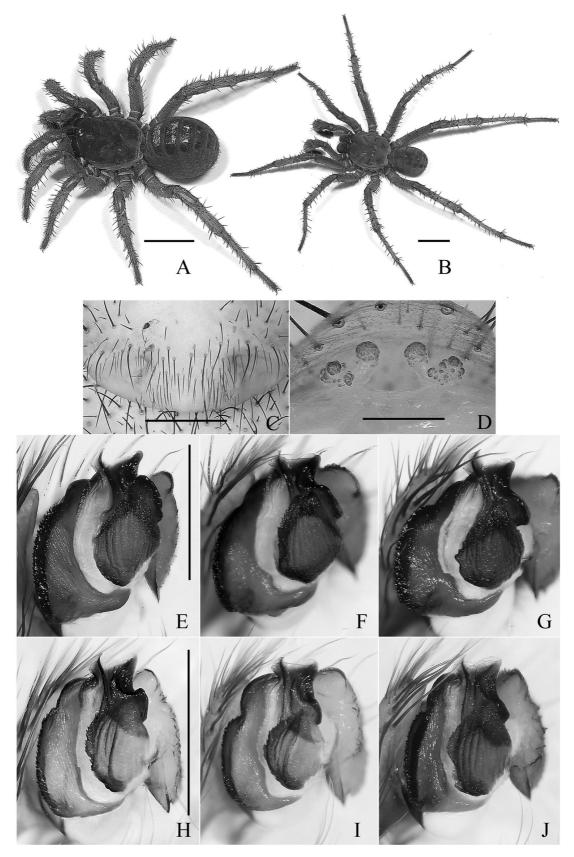


Fig. 4. Heptathela helios n. sp. (A-G) and H. yanbaruensis (H-J). A, habitus of female (paratype from Benoki, NSMT-Ar 12858); B, habitus of male (holotype from Oku, NSMT-Ar 12851); C, female genital area, ventral view (paratype from Benoki, NSMT-Ar 12858); D, spermathecae, dorsal view (paratype from Ada, NSMT-Ar 12861); E-G, male palp, distal view (E: holotype from Oku, NSMT-Ar 12851; F-G: pratypes from Oku, NSMT-Ar 12855; H-J: from Nago). Scales = 5 mm (A-B), 1 mm (C-F).

12851, Fig. 3B) and one of female paratypes (NSMT-Ar 12858, Fig. 3A). Coloration and markings in alcohol. Female and male. Carapace pale brown, mottled with dark brown, head region anteriorly darker. Abdomen pale brown, tergites dark brown.

Measurements. 3/9, measurements in parentheses indicate the range among type series. Body 12.77 (10.15–13.38)/14.62 (10.31–14.62) long. Carapace 5.91 (5.13–6.13)/6.50 (4.69–6.50) long; 5.13 (4.63–5.50)/5.80 (4.38–5.80) wide. Length of legs [tarsus + metatarsus + tibia + patella + femur = total]: I, 2.63 + 5.00 + 3.84 + 2.38 + 5.38 = 19.20 / 1.69 + 3.00 + 2.81 + 2.63 + 4.91 = 15.04; II, 3.00 + 5.31 + 3.69 + 2.38 + 5.19 = 19.57/1.88 + 3.13 + 2.56 + 2.63 + 4.69 + 14.89; III, 3.50 + 6.25 + 3.50 + 2.44 + 5.00 = 20.69 / 2.13 + 3.63 + 2.50 + 2.63 + 4.44 = 15.33; IV, 4.19 + 8.19 + 5.00 + 2.66 + 6.69 = 26.73 / 3.06 + 5.88 + 3.88 + 3.00 + 6.31 = 22.13. Abdomen 5.13 (4.25–6.06)/8.25 (4.81–8.25) long, 3.88 (3.13–4.69)/6.10 (3.25–6.30) wide.

Carapace longer than wide [length / width 1.15 (1.08–1.15)/1.12 (1.07–1.22)]. Eye area wider than long [length/width 0.85 (0.83–0.88)/0.79 (0.79–0.90)]. Fang furrow of chelicera with 12 (7–12)/13 (11–13) anterior marginal teeth and no retromarginal teeth. Labium wider than long [length/width 0.48 (0.38–0.50)/0.53 (0.48–0.53)]. Sternum longer than wide [length/width 2.15 (1.78–2.18)/1.92 (1.65–2.00)]. Length of leg I/length of carapace 3.25 (3.16–3.39)/2.31 (2.07–2.34). Male palp as in Fig. 4E–G. Abdomen longer than wide [length/width 1.32 (1.29–1.36)/1.35 (1.27–1.48)], female genital area and spermatheca as in Fig. 4C–D.

Distribution. Northernmost part of Okinawajima Is., Nansei Isls., Japan.

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Appendix. Localities, haplotype or genotype names, and DDBJ/EMBL/GenBank accession numbers of sequence data analyzed in the study. *Heptathela* sp. is described as *Heptathela helios* n. sp. in this paper. A and B indicate the sympatric localities corresponding to Fig. 1.

Trepramera sp. is described as		COI					
Locality	N	Haplotype	Accessin No.	28S Genotype	Accession no.	H3 Genotype	Accession no.
Heptathela sp.							
N26.85868 E128.26139	1	OKHH05	AB918042	_	_	_	_
N26.85868 E128.26139	1	OKHH09	AB918045	_	_	_	_
N26.84274 E128.28737	1	OKHH13	AB918049	_	_	_	_
N26.84120 E128.28388	1	OKHH26	AB918062	_	_	_	_
N26.83839 E128.25859	1	OKHH04	AB918041	<u> </u>	<u> </u>	<u> </u>	<u> </u>
N26.83633 E128.27544	1	OKHH10	AB918046	_	_	_	_
N26.83532 E128.28658 N26.83532 E128.28658	2 1	OKHH06 OKHH07	AB918043 AB918044	_			
N26.83436 E128.30238	2	OKHH07 OKHH02	AB918039	_	_	_	_
N26.83436 E128.27615	1	OKHH20	AB918056	_	_	_	_
N26.81580 E128.30493	5	OKHH15	AB918051	_	_	_	_
N26.81580 E128.30493	1	OKHH23	AB918059	_	_	_	_
N26.81286 E128.31701	1	OKHH03	AB918040	_	_	_	_
N26.81286 E128.31701	1	OKHH11	AB918047	_	_	_	_
N26.81254 E128.29852	1	OKHH17	AB918053	_	_	_	_
N26.80313 E128.29236	1	OKHH01	AB918038	_	_	_	_
N26.79985 E128.26064	3	OKHH14	AB918050	_	_	_	_
N26.79985 E128.26064	1	OKHH18	AB918054	_	_	_	_
N26.79985 E128.26064	2	OKHH19	AB918055	_	_	_	_
N26.79797 E128.28802	2	OKHH16	AB918052	_	_	_	_
A: N26.77713 E128.26423	1	OKHH25	AB918061	OKHH2801	AB918102	HH02	AB918106
B: N26.75557 E128.30072	1	OKHH12	AB918048	OKHH2801	AB918102	HH01	AB918105
B: N26.75557 E128.30072	1	OKHH21	AB918057	OKHH2801	AB918102	HH04	AB918108
B: N26.75557 E128.30072	1	OKHH22	AB918058	OKHH2801	AB918102	HH03	AB918107
B: N26.75557 E128.30072	1	OKHH24	AB918060	OKHH2801	AB918102	HH04	AB918108
Heptathela yanbaruensis							
N26.83633 E128.27544	1	OKHY203	AB778252	_	_	_	_
N26.81074 E128.23613	4	OKHY114	AB918075	_	_	_	_
N26.79795 E128.25816	9	OKHY114	AB918075	_	_	_	_
N26.79303 E128.31055	4	OKHY104	AB918066	_	_	_	_
N26.78692 E128.22362	2	OKHY202	AB918097	——————————————————————————————————————	A D010102		A D010110
A: N26.77713 E128.26423 A: N26.77713 E128.26423	1 1	OKHY203 OKHY203	AB778252 AB778252	OKHY2809 OKHY2801	AB918103 AB778049	HY02 HY06	AB918110 AB918114
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A: N26.77713 E128.26423	1	OKHY203	AB778252	OKHY2801	AB778049	HY15	AB918123
A: N26.77713 E128.26423	1	OKHY205	AB918099	OKHY2801	AB778049	HY09	AB918117
A: N26.77713 E128.26423	1	OKHY205	AB918099	OKHY2810	AB918104	HY01	AB918109
A: N26.77713 E128.26423	1	OKHY206	AB918100	OKHY2801	AB778049	HY08	AB918116
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N26.77398 E128.31318	4	OKHY103	AB918065	_	_	_	_
N26.76707 E128.25806	2	OKHY204	AB918098	_	_	_	_
N26.761207 E128.218956	6	OKHY121	AB918082	_	_	_	_
N26.761207 E128.218956	3	OKHY124	AB918085	_	_	_	_
N26.761207 E128.218956	1	OKHY125	AB918086	_	_	_	_
B: N26.75557 E128.30072	2	OKHY101	AB918063	OKHY2801	AB778049	HY03	AB918111
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY04	AB918112
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY10	AB918118
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY11	AB918119
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY12	AB918120
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY13	AB918121
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY14	AB918122
B: N26.75557 E128.30072	3	OKHY101	AB918063	OKHY2801	AB778049	HY16	AB918124
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY17	AB918125
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY18	AB918126
B: N26.75557 E128.30072	15	OKHY101	AB918063	OKHY2801	AB778049	HY19	AB918127
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY20	AB918128
B: N26.75557 E128.30072	2	OKHY101	AB918063	OKHY2801	AB778049	HY21	AB918129
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY22	AB918130
B: N26.75557 E128.30072	2	OKHY101	AB918063	OKHY2801	AB778049	HY23	AB918131
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY24	AB918132
B: N26.75557 E128.30072 B: N26.75557 E128.30072	11 1	OKHY101 OKHY101	AB918063 AB918063	OKHY2801 OKHY2801	AB778049 AB778049	HY25 HY26	AB918133 AB918134
D. 1120./333/ E120.300/2	1	OK111101	AD710003	OK1112001	AD / / 0047	11120	AD710134

B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY27	AB918135
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY29	AB918137
B: N26.75557 E128.30072	1	OKHY101	AB918063	OKHY2801	AB778049	HY28	AB918136
N26.75438 E128.24580	3	OKHY204	AB918098	—	—	—	—
N26.74856 E128.23824	6	OKHY123	AB918084	_	_	_	_
N26.74845 E128.23832	2	OKHY123	AB918084	_	_	_	_
N26.74133 E128.28724	4	OKHY115	AB918076	_	_	_	_
N26.73838 E128.23784	1	OKHY123	AB918084	_	_	_	_
N26.73838 E128.23784	5	OKHY201	AB918096	_	_	_	_
N26.73382 E128.22142	2	OKHY201	AB918096	_	_	_	_
N26.72310 E128.17779	1	OKHY126	AB918087	_	_	_	_
N26.72310 E128.17779	3	OKHY127	AB918088	_	_	_	_
N26.71531 E128.22219	2	OKHY122	AB918083	_	_	_	_
N26.71531 E128.22219	1	OKHY124	AB918085	_	_	_	_
N26.71531 E128.22219	2	OKHY201	AB918096	_	_	_	_
N26.70522 E128.26914	1	OKHY102	AB918064	_	_	_	_
N26.69134 E128.10928	2	OKHY108	AB918070	_	_	_	_
N26.69134 E128.10928	2	OKHY109	AB918071	_	_	_	_
N26.67941 E128.24016	2	OKHY117	AB918078	_	_	_	_
N26.66930 E127.95393	4	OKHY131	AB918092	_	_	_	_
N26.66930 E127.95393	1	OKHY134	AB918095	_	_	_	_
N26.65080 E128.15886	2	OKHY119	AB918080	_	_	_	_
N26.65080 E128.15886	2	OKHY120	AB918081	_	_	_	_
N26.64698 E128.20703	4	OKHY116	AB918077	_	_	_	_
N26.64056 E128.23965	1	OKHY118	AB918079	_		_	
N26.62611 E127.99059	2	OKHY107	AB918069	_	_	_	_
N26.62398 E128.06210	1	OKHY110	AB918072	_	_	_	_
N26.62398 E128.06210	1	OKHY111	AB918073	_	_	_	_
N26.61970 E127.91851	1	OKHY113	AB918074	_	_	_	_
N26.61563 E128.09049	3	OKHY112	AB778251	_	_	_	_
N26.61563 E128.09049	3	OKHY130	AB918091	_	_	_	_
N26.58921 E127.99792	7	OKHY105	AB918067	_	_	_	_
N26.58180 E128.01041	2	OKHY105	AB918067	_	_	_	_
N26.58180 E128.01041	2	OKHY106	AB918068	_	_	_	_
N26.55679 E128.00909	1	OKHY132	AB918093	_	_	_	_
N26.55508 E128.05146	3	OKHY128	AB918089	_	_	_	_
N26.55508 E128.05146	1	OKHY129	AB918090	_	_	_	_
N26.53760 E127.96842	1	OKHY133	AB918094	_	_	_	_